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Physiochemical Food System Studies

2nd Quarterly Report

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No restrictions requested for this report.

ABSTRACT

Funds and work level have been expanded in this quarter. Four feeding studies have shown that administration of formose as a solution is feasible, and that most toxic effects observed are reversible after cessation of treatment. Two formulations of formose have been administered; "formose C" is more toxic than "formose LBM-3". Age or weight at the initiation of treatment appears to be a significant variable. Data analysis techniques are being expanded and data from formose work in other laboratories will be analyzed with the system developed.

GENERAL

In this second quarter we have reassessed and reorganized the project in accordance with the increased work to be performed, and have received additional funds for this change. We have completed four feeding studies and have another in progress; the results have been partially analyzed. We have undertaken to create a data analysis system to include our work and that of other related programs under Dr. Shapira, the technical monitor. Portions of this system which have been completed have been used on our experimental data. The cooperation with Dr. Shapira at the Ames Labs has been increased, and all chemical characterization of formose sugars by I.C.B. personnel has been transferred to that lab.

PROJECT REORGANIZATION

The basic research goals of the project remain the same: the characterization of the content and toxicity of formose. Work under this project has been reassigned in order to better coordinate with other projects working with Dr. Shapira on various aspects of the formose problem.

Formose toxicity is being explored with animal diet studies performed at I.C.B. This is presently centered around feeding formose sugars and other carbohydrates as liquid solutions. This route of administration is open to change should the need arise.

The chemical content of formose sugars is being investigated at the Ames Laboratories by I.C.B. personnel working very closely with Dr. Shapira. Gas chromatography is presently the principal technique in use.

The project has been expanded to include development of a data analysis system. Because of the availability of trained personnel and equipment, I.C.B. is designing the software for the analysis of data generated in its own experiments and in those of other laboratories under Dr. Shapira's technical direction on the formose feeding projects. Thus a consistent technique of analysis will be used for several projects.

CHEMICAL CHARACTERIZATION OF FORMOSE MIXTURES

During the previous quarter several types of chromatography were considered for analysis of formose mixtures, and gas-liquid chromatography (GLC) was considered the most fruitful. During this quarter GLC techniques have been further developed in Dr. Shapira's laboratories at Ames, and some applications of this technique to metabolic products have been explored. We have supplied some technical manpower in this work. As of July 1, 1969 we are supplying full time a Ph.D. chemist to expand this work and coordinate several aspects of the problem of chemical characterization.

DATA REDUCTION

Data reduction was originally performed on an Olivetti Programma 101 desk-top computer but is was later transferred to an IBM 1130 system with a Calcomp plotter. Since the expansion of the project, we are continuing with the 1130 procedures, but are also designing procedures to be used with an IBM system 360 at NASA Ames Laboratories. These will be applied to results of several projects under Dr. Shapira's supervision.

The data presented in this report has been analyzed with the programs completed. Other analysis will be done as programming is expanded, particularly when the larger computational area of the System 360 can be realized.

The principal measure of toxicity is presently the slope of a linear regression line on the weights of animals. For short equal periods the growth of rats of a given species, sex and birth date is sufficiently linear to be modeled linearly. This measure is not satisfactory over long periods, and will be succeeded by a correlation to a curvilinear model of growth. We are working toward a measure which will also allow comparison between species.

For this report, most of the data is presented in the form of graphs of daily group average weights. These are duplicated directly from the plotter output. Other plotting routines are being prepared for Dr. Shapira's needs.

DIET STUDIES

During this quarter we have completed three diet studies and initiated others. The data from these have been partially analyzed. Experiment 1, reported previously, is again described because of its relevance to later studies. Questions examined have included the feasibility of feeding formose and other carbohydrates as solutions, the relative toxic effects of two different formose mixtures, and the effects of the age of the animals on observed toxicity of formose. The toxicity of formose has been observed in reduced or reversed weight gain, diarrhea, and gross morphologic change of organs. The first two of these changes are reversible.

GENERAL

The animals used are Sprague-Dawley derived rats bred by Simonsen Laboratories. This strain was selected because the high growth amounts allow better determination of rate, and because it is a more hardy strain than some inbred ones. This makes it more suitable to studies of the reversible, sub-acute toxicity we are presently observing. Only males have been used, to avoid sex difference in response. Later work will expand data into females, generation studies, and other strains. Ultimately, of course, studies of other

species must preceed human studies of non-toxic formose-derived carbohydrates.

Although experiments vary, a general regimen is followed. The experimental mixtures are administered ad lib as solutions. This allows carbohydrate intake to be calculated. Tap water is used as a control. A standard block rat chow ("Lab-Blox") is also administered ad lib, and consumption is measured. From this we will calculate dietary intake of other materials and of non-experimental carbohydrate. Weight change is measured as the principal indicator of toxic effect. Levels of formose used so far are not acutely toxic.

Measurements are taken at various time intervals on the basis of the needs of experimental evaluation and statistical need. Monday-Wednesday-Friday measurements, allowing a maximum of three days between measurements, are the least frequest schedule used. Daily measures are taken when first evaluating a material in order to prevent irreversible degradation of the animal. Most experiments allow the experimental animals to be used as self-controls by changing from an experimental condition to a control one, or the reverse. Acute toxicity will be investigated if needed.

For convenience in data reduction, experiments are broken into several phases for the different carbohydrate

administration regimens. A two or three day acclimation period is often defined at the beginning of a phase to allow the animals to learn the new dietary and feeding environment. This approach is to isolate metabolic changes from behaviorally induced changes. This will be re-evaluated, when there is more data, by comparison with the same information without this subdivision.

Experiment 1:

Six litter mates 46 days old were started on phase one. Three were administered 5% "formose C" obtained from Dr.Shapira; three were controls. The supply of formose was exhausted in 8 days, and one animal from each group was sacrificed for autopsy. The remaining experimental animals were returned to tap water, phase 2, for 15 days, giving an experiment of 23 days length.

During phase 1 the experimental enimals lost weight (Fig. 1) and were diarrhetic while the controls grew at a rate judged normal. (Determination of the normal growth rate of this strain in our laboratories is in progress) The one experimental animal autopsied had gross G.I. tract abnormalities including inflammation and low food content. These were not exhibited by the control.

During phase 2 the experimental animals, now on water, grew at a faster rate than the controls, but did not equal the controls in absolute weight before determination. Autopsies of all these animals showed none of the irregularities seen in the earlier case.

From this experiment it was concluded that liquid administration was a satisfactory technique. It was also apparent that "formose C" has toxic or deleterious effects which were reversible. This is regarded as a strong indication that the toxicity is of a nature to allow a useable

food carbohydrate to be made from"formose C" with adequate preparation and purification. The last day of the experimental phase showed a slight upturn of weight which was regarded as a possible indicator that the animals in some way were adapting to the formose. This raises a question for further investigation.

Experiment 2:

Sixteen male rats 33 days old were started on phase 1. For 14 days they were all allowed ad lib food and water, and consumption of these and weights were measured daily. They were then divided into four groups by weight matching. There were no abnormal weight changes for the day. For phase 2, the four groups were administered the following liquids ad lib: tap water (control group), 2.5% "formose LBM-3 (obtained from Dr. Shapira), 5% "formose LBM-3", and 5% glucose (Dextrose, Hydrous, USP, J.T.Baker). These groups were numbered 1, 2, 3 and 4, respectively. Measurements were continued daily until day 37. Phase 3 was then initiated: group 1 was changed from water to 5% "Formose LBM-3; group 2 was terminated and autopsied; and groups 3 and 4 were changed to tap water from 5% "formose LBM-3" and 5% glucose, respectively. Measurements were continued daily for 13 days. All animals were terminated and autopsied at 83 days of age.

During phase 1 all animals grew at the same rate (Fig. 2), precluding the interpretation that later differences could be due to selection of animals. Table 1 shows the slopes of the linear regression lines of growth for the separate phases, and of the correlation of these lines. (At this point, the slopes are best considered as guiding data when observing the graph, as no standards of comparison have yet been determined. The very high correlation coefficients result in part from calculation based on mean values.)

After administration of the experimental liquids in phase 2, some differences between the water controls and the experimental groups become noticable. The growth rate of all experimental animals was greater than that of the water animals. However, group 3, on 5% "formose-LBM-3", had a short period of three days with a lower rate of growth. Thereafter, their actual weights were lower than those of the other groups. This was not observed in group 2 (2.5% "formose LBM-3") and was much less severe in effect than observed in the previous experiment with another formulation of formose. The higher growth rates (Fig. 4) were accompanied with higher fluid intakes, (Fig. 3) leading to the possibility that these animals might retain higher percentages of tissue fluid. As no measurement of tissue fluid or urine output were made, and correlations of weight to fluid intake have not yet been made, the greater growth rate cannot be ascribed clearly to the calorific content

of the experimental diets. Diarrhea was observed in all "formose LBM-3" treated animals.

Phase 3 was initiated to examine the reversibility of the phase 2 effects. When group 2 was autopsied, all organs were normal except that the G.I. tract contained less material than normal. Among the other three groups, the change in treatment produced noticeable effects which were reasonable in the light of previous data. Overall, the growth rates were lower than previously observed, due to the non-linearity of growth over a long period. The growth rate of group 3 was greater than that of group 4, although the absolute weights were lower. This was interpreted as an indication that the growth in the previous period was abnormal, as previously mentioned, and that the animals were returning to a more normal condition: group 3 was "regaining" losses in absolute weight sustained in the accommodation period when first administered formose, while group 4 was "losing" its glucose-induced absolute gains. Group 1, changed from tapwater to formose, showed losses expectable with the more toxic diet. However, this group did not show a clearly defined accommodation period; the toxicity in this period was exhibited by a growth rate lower than either of the other groups. This raised the question of possible effects of age upon the accommodation response to "formose LBM-3".

The remaining animals were sacrificed and autopsied at the end of the experiment. In group 1 all animals showed slightly enlarged and empty G.I. tracts; one liver showed enlargement and another granulation. In group 3 three animals showed slightly enlarged G.I. tracts which were normally full; all showed slight liver enlargement. These observations further support the growth weight observations that the toxic effects of "formose LBM-3" are reversible, and hint at a toxic mechanism involving G.I. tract absorption. In group 4 all G.I. tracts were normal in size and content, but two showed signs of kidney granulation. Tissues from all animals were preserved in formalin for later examination.

Experiment 3:

Experiment 1 and 2 showed a marked difference in response to formose feeding. Different formulations of formose had been used, but also age of the animals at their first exposure to formose was also different. Experiment 3 was designed to throw some light on the specific effects of these variables. As the supply of "formose C"was exhausted, only age could be controlled. It was decided to expose four groups, differing in age by one week, to "formose LBM-3", of which there was sufficient supply.

Twenty male rats were used, in four groups of litter mates. Phase 1, feeding 5% "formose LBM-3" solution and rat chow ad lib, was started immediately and continued for 21 days. In phase 2 the formose was replaced with tap water, and continued for 9 days, giving a total experiment length of 30 days. Weight, and food and liquid intakes were measured.

Table 2 shows the growth rates in this experiment. With the exception of group 1, all animals grew at higher rates when fed "formose LBM-3" than when later fed water. This is attributed to gradual reduction in growth rate that is normal over this age range. Correlation of this data to standardized growth curves has not yet been done, so no conclusion can be drawn at this point regarding the toxicity exhibited. A graph of the daily average weights of these groups (Fig.4) does indicate a slight toxic effect. Groups 3 and 4 both lost weight for a short period after being started on the formose diet.

Examination of the weights and ages of the animals at the start of this experiment brought attention to a new variable: the rate of growth of Sprague-Dawley derived rats varies in an annual cycle, with weights at a given age increasing during the spring months. A procedure for normalizing data will be determined. In the meantime, a rough measure was adopted to determine the effects of age, and

therefore of formose formulation fed. Data concerning the growth of all groups of animals fed 5% formose was ordered by age and by weight (Tables 3 and 4 respectively) at initiation of formose treatment. It is clear that the "formose-C" treated animals were more toxically affected than those treated with "formose LBM-3", although there is no clear age or weight effect at this point.

Autopsies of these animals, after the 9 day period to allow reversal of the toxic effects, showed enlargement of the G.I. tract of varying degrees in most cases. Three showed signs of hemorrhage in the G.I. tract, four showed slight enlargement of the liver, and two had enlarged kidneys. These effects will be considered in greater detail as more data are gathered.

Experiment 4:

Experiment 4 was initiated to determine the utility of grouping animals for measurement rather than measuring individually. Five males, 38 days old, were caged together and treated with tap water -- control condition. Measurements were made daily of weight and of food and liquid intake.

Shortly after initiation of this experiment, discussion with the scientific monitor showed that grouped measurements would be incompatible with other experiments concerning the toxic effects of formose. Therefore, the experiment was terminated after 18 days. The data will be considered in

determining normalized growth rates for the rats we are using.

ADMINISTRATIVE

The funds for expansion of the work, requested in March, have been received. One Ph.D. chemist has been engaged to work at NASA Ames Laboratoriesdirectly with Dr. Shapira on problems of chemical formulation of formose sugars. A slight rebudgeting of funds has been allowed in order that we may do some complex computer work requiring the use of an IBM system 360; this will not affect the University's contribution of its own computer as originally planned.

Table 1

Experiment 2

GROWTH RATES, SLOPE OF LINEAR REGRESSION

GROUP	PHASE	TREATMENT	SLOPE	CORRELATION
1	1	H_2O	7.37	0.99
1	2	H_2O	5.95	0.99
1	3	5% formose	2.78	0.98
2	1	H_2O	7.70	0.99
2	2	2.5% formose	6.81	0.99
2	3	*	*	*
3	1	H_2O	7.53	0.99
3	2	5% formose	6.82	0.99
3	3	H_2O	4.79	0.98
4	1	H_2O	7.27	0.99
4	2	5% glucose	6.23	0.99
4	3	H_2O	3.69	0.98
comb.**	1	H_2O	7.46	0.96

* terminated at end of phase 2

** all animals, phase 1

Table 2

Experiment 3

GROWTH RATES, SLOPE OF LINEAR REGRESSION

GROUP	PHASE	TREATMENT	SLOPE	CORRELATION
1	1	formose	6.68	0.99
2	2	H_2O	9.23	0.99
2	1	formose	7.78	0.99
2	2	H_2O	7.75	0.98
3	1	formose	6.62	0.99
3	2	H_2O	5.65	0.99
4	1	formose	5.73	0.99
4	2	H_2O	5.51	0.94

Table 3

5% FORMOSE TREATED ANIMALS

ORDERED BY AGE

AGE	GROUP	WEIGHT	FORMOSE EFFECT
22	3-1	55	Short reduced rate of growth
29	3-1	88	Short reduced rate of growth
36	3-3	149	Short loss of weight
43	3-4	182	Short loss of weight
46*	1-1	124	Continual loss of weight
48	2-3	189	Reduced rate of growth
71	2-1	325	Short reduced rate of growth

* "formose-C" treated - all others treated with "formose LBM-3"

Table 4

5% FORMOSE TREATED ANIMALS

ORDERED BY WEIGHT

WEIGHT, g.	GROUP	AGE, days	FORMOSE EFFECT
55	3-1	22	short reduced rate of growth
88	3-2	29	short reduced rate of growth
124*	1-1	46	continual weight loss
149	3-3	36	short weight loss
182	3-4	43	short weight loss
189	2-3	48	short reduced rate of growth
325	2-1	71	reduced rate of growth

* "Formose-C" treated - all others treated with "Formose LBM-3"

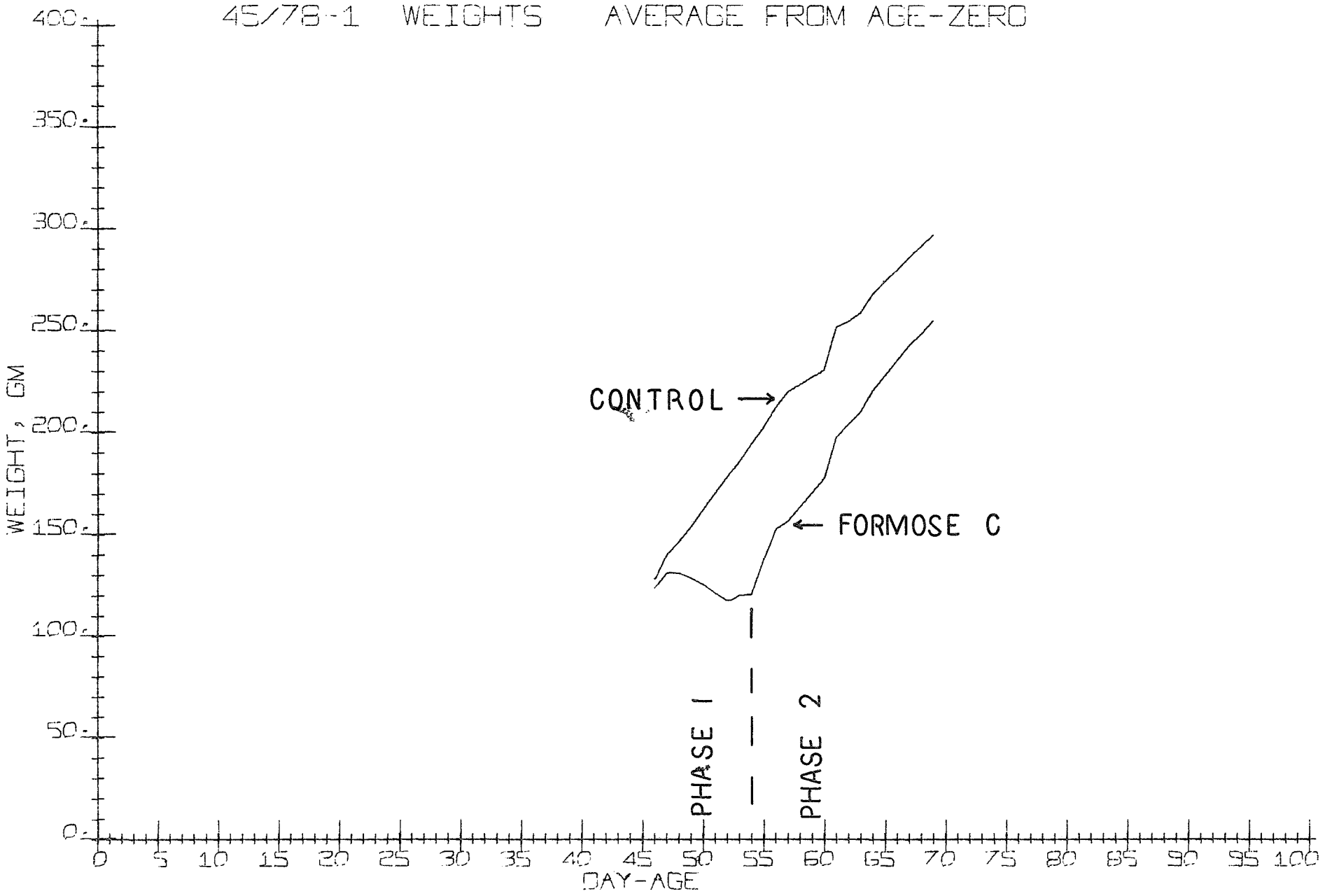
45/78-1 WEIGHTS AVERAGE FROM AGE-ZERO
WEIGHT, GM
400
350
300
250
200
150
100
50
0
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100
DAY-AGE
CONTROL
FORMOSE C
PHASE 1
PHASE 2

FIG 1.

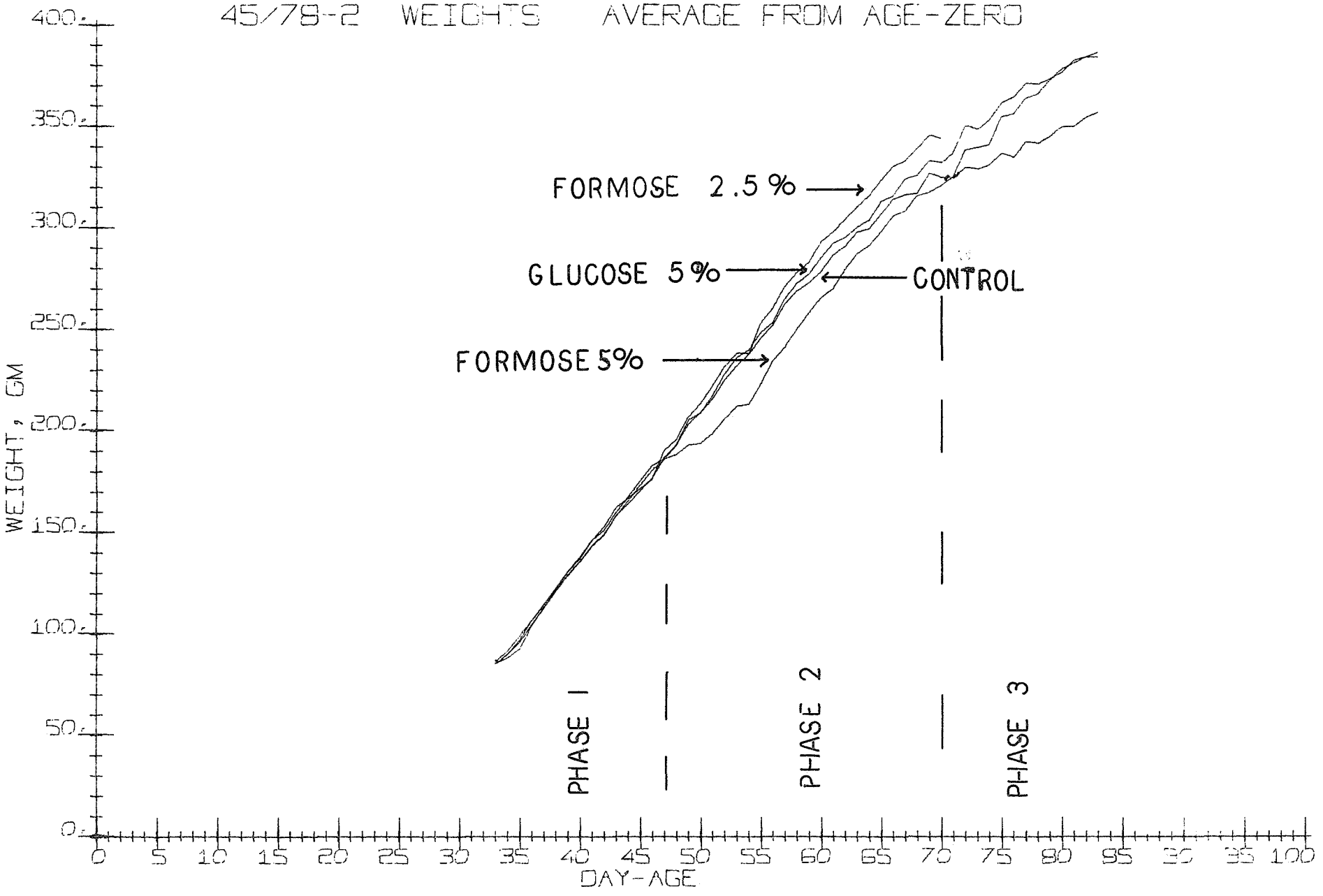
45/78-2 WEIGHTS AVERAGE FROM AGE-ZERO
WEIGHT, GM
400
350
300
250
200
150
100
50
0
FORMOSE 2.5 %
GLUCOSE 5%
FORMOSE 5%
CONTROL
PHASE 1
PHASE 2
PHASE 3
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 95 35 35 100
DAY-AGE

FIG 2.

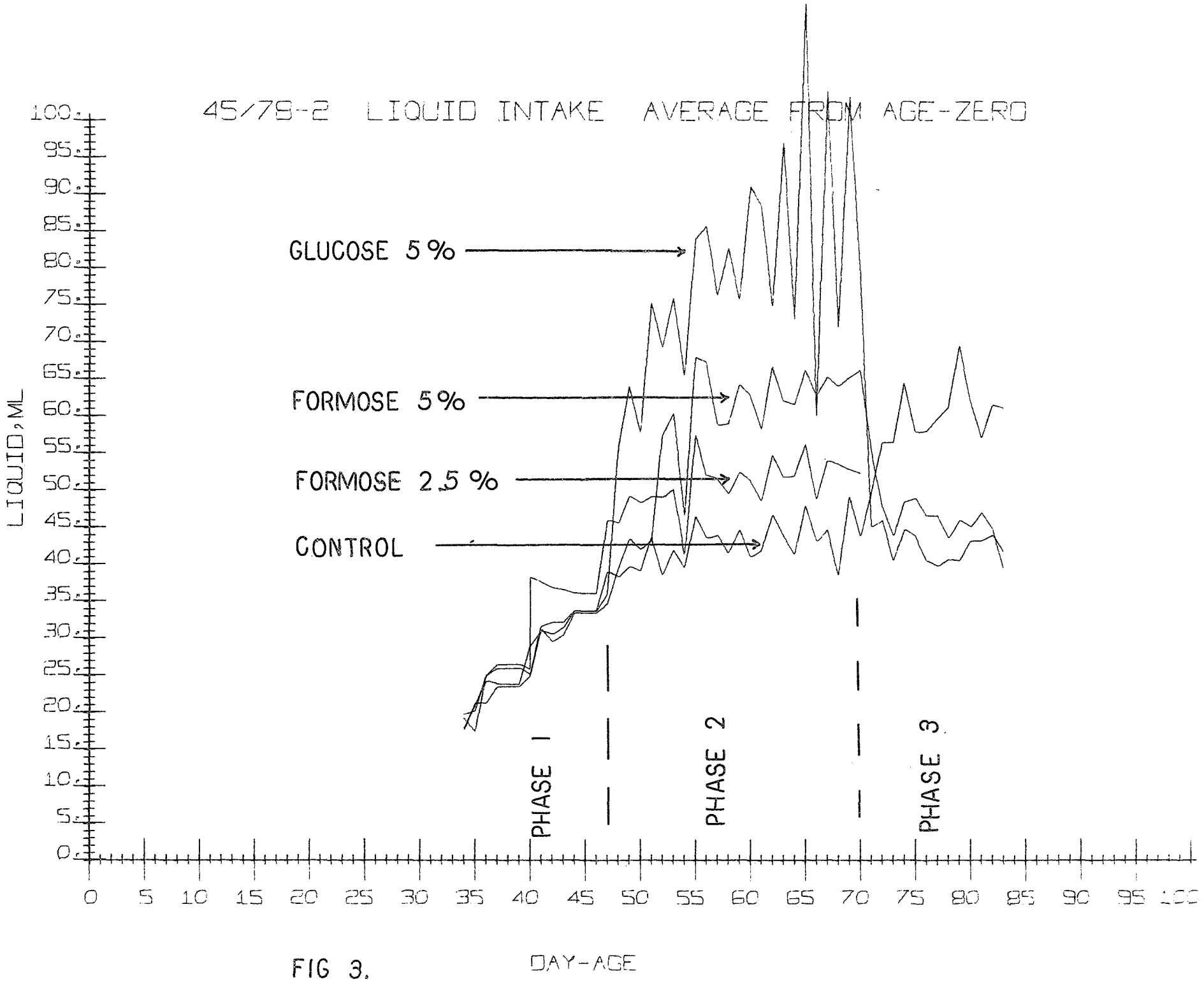
45/78-2 LIQUID INTAKE AVERAGE FROM AGE-ZERO
GLUCOSE 5%
FORMOSE 5%
FORMOSE 2.5%
CONTROL
PHASE 1
PHASE 2
PHASE 3
LIQUID, ML
DAY-AGE
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100

FIG 3.

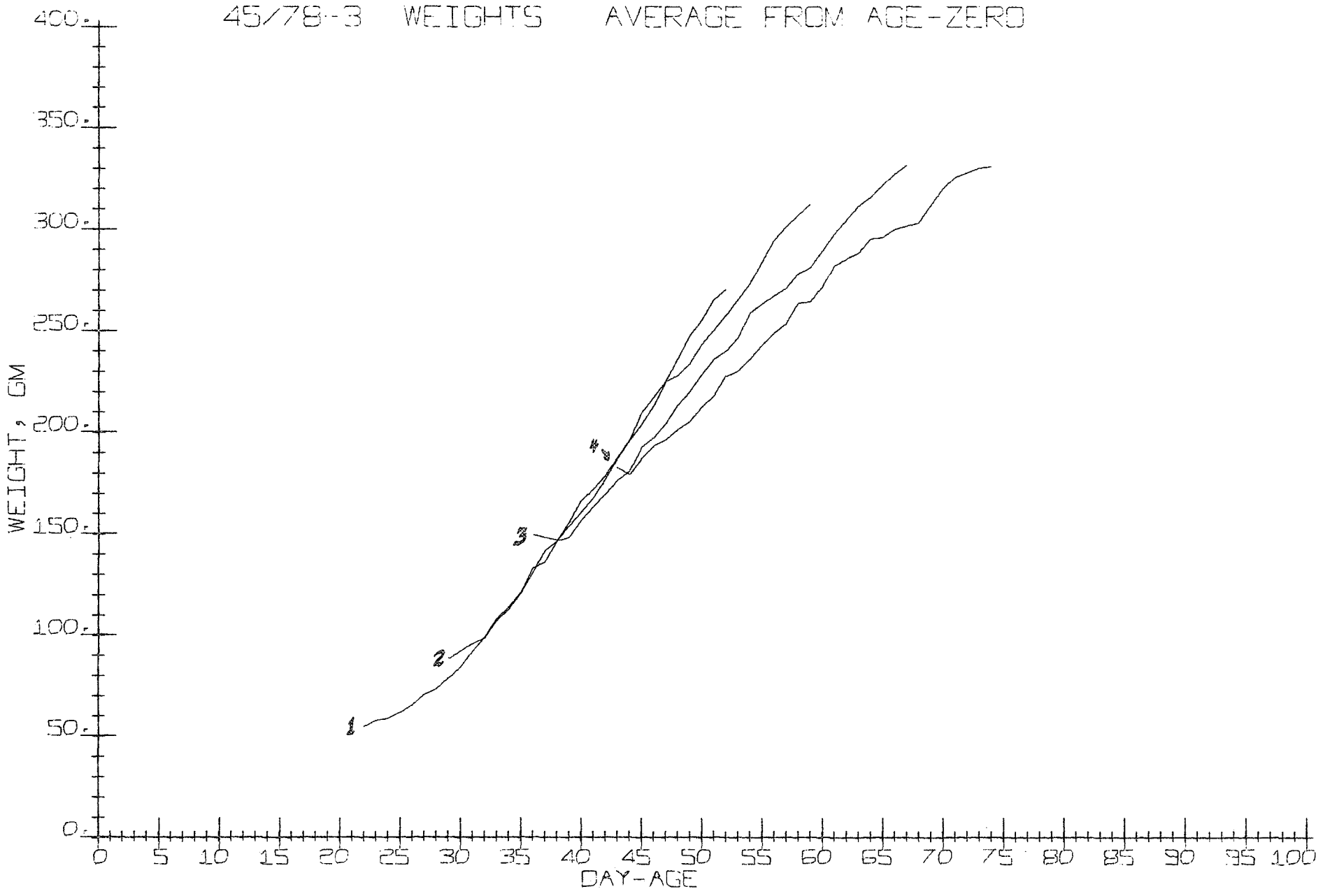
45/78-3 WEIGHTS AVERAGE FROM AGE-ZERO
WEIGHT, GM
400.
350.
300.
250.
200.
150.
100.
50.
0.
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100
DAY-AGE
1
2
3
4

FIG 4.